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The population structure of planktonic protists and small metazoans in Mali Ston Bay (Adriatic Sea) - implications for determination of trophic state and shellfish culturing potential

Frano KRŠINIĆ^{1*}, Marijeta ČALIĆ² and Marina CARIĆ²

¹*Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia*

²*University of Dubrovnik, Institute for Marine and Coastal Research, Kneza Damjana Jude 12, 20000 Dubrovnik, Croatia*

**Corresponding author, e-mail: fkrsinic@izor.hr*

Samplings in the Bay Mali Ston were performed between January 2002 and January 2003 at station Usko, conducted either once a week or twice a month, with 5-L Niskin water sampler at 1 m depth intervals from the surface to 13 m. The investigations of the population of planktonic protists and small metazoans covered the major assemblages: taxopodids, non-loriccate ciliates, tintinnids, rotatorians, copepod nauplii, calanoids, oithonids, oncaeids, harpacticoids, bivalve larvae and juvenile appendicularians. The diversity and abundance of all assemblages on an annual basis is primarily dependent on variations in the hydrographic conditions. During periods of low temperature and high salinity, oncaea copepodites and adults show very high abundance, and together with non-loriccate ciliates, tintinnids and planktonic harpacticoids form the winter-spring zooplanktonic community. During the warmer period in conditions of higher temperature and salinity the summer-autumn zooplanktonic communities comprise high abundances of post-naupliar calanoids and oithonids, juvenile appendicularians and summer tintinnids. From the abundance of planktonic protists and small metazoans it can be concluded that the Bay Mali Ston has meso-oligotrophic characteristics. This paper discusses the importance of the bio-deposition of faecal material from the shellfish that are cultured in the Bay and the potential negative impact on the ecological balance resulting from any significant increased capacity in aquaculture.

Key words: Adriatic Sea, Mali Ston Bay, distribution, zooplankton

INTRODUCTION

Mali Ston Bay is located between a south-east-northwest oriented coast and Pelješac, the largest peninsula in the Middle Adriatic (Croatia). The bay is narrow, approximately 5 km wide, with a greatest depth of 29 m in the

middle region of the outer bay. Its geomorphologic character, specific hydrographical and production features make it unique along the eastern Adriatic coastline. The effects of open seawaters on the bay are strongest during the winter season when highest salinity occurs due to the intrusion of bottom currents. Furthermore,

during winter, the surface layers are mixed with fresh waters from the many springs from the mainland and the peninsula side of the bay. The Neretva River occasionally influences Mali Ston Bay, and the effects, when they occur, are very strong. Accordingly, the position of Mali Ston Bay on the eastern shores of the Adriatic is very favourable, as the interactions of the water masses with various features give rise to optimal conditions for high productivity. The increase in biological diversity is manifested through the number of species and population density of benthic filter feeders, for example, the natural populations of shellfish, stone coral, polychaetes, tunicates, small blue fish, mussels and commercially farmed oysters. A number of complex investigations were carried out regularly at the beginning of the 1970 on a few stations throughout Mali Ston Bay, for the purpose of its protection. The hydrographical and productivity conditions were analysed BULJAN *et al.*, 1973; MARASOVIĆ & PUCHER-PETKOVIĆ, 1981; VILIČIĆ, 1981; VUČAK *et al.*, 1981; VUKADIN, 1981. Smaller zooplankton were investigated by KRŠINIĆ, 1979, 1980, 1987a,b; KRŠINIĆ & MUŠIN, 1981; RUDENJAK-LUKENDA, 1990, while crustaceans and other mesozooplanktonic organisms: BULJAN *et al.*, 1973; MUŠIN, 1986; ONOFRI, 1986; LUČIĆ & ONOFRI, 1990; VILIČIĆ *et al.*, 1994; LUČIĆ & KRŠINIĆ, 1998. Frequent investigations of the water column were performed between January 2002 and January 2003, and the first complex qualitative-quantitative data of planktonic protozoans and small metazoans are presented in this paper. Zooplankton data were also used as a basis for determination of the trophic condition within the specific environment of the Mali Ston Bay, and the related potential for the aquaculture activities is discussed.

MATERIAL AND METHODS

Samples for physico-chemical, phytoplankton and zooplankton analysis were obtained during the period 18 January 2002 to 31 January 2003 at Usko station (Fig.1). From January to May sampling was conducted once a week, and from June to December mostly twice a month.

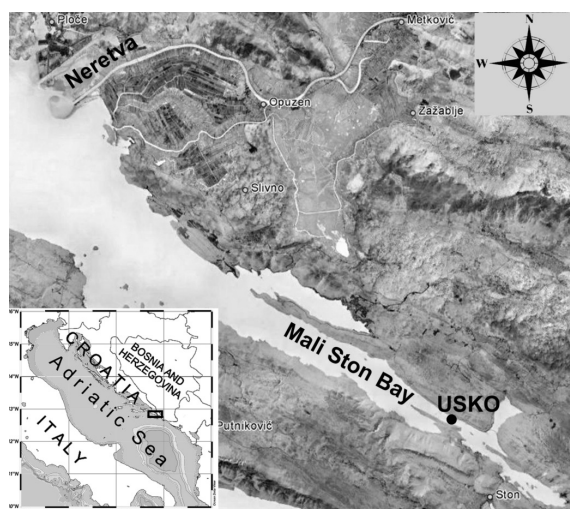


Fig. 1. Location of sampling station Usko in the Bay of Mali Ston.

Water samples were taken with 5-L Niskin water sampler at 1 m depth intervals from the surface to 13 m. A total of 33 fieldworks were carried out and 395 samples of physico-chemical (temperature, salinity, dissolved oxygen and nutrients) and biological parameters (phytoplankton and zooplankton abundances, and phytoplankton biomass expressed as chlorophyll *a*) were processed.

Water temperature was measured with an inverted thermometer. Salinity was determined by the standard Mohr-Knudsen titration method with silver nitrate. Density (σ_t) was calculated from temperature and salinity at given depths, based on the standard equation. Oxygen saturation (O_2/O_2') was calculated from the solubility of oxygen in seawater as a function of the temperature and salinity. Transparency has been measured as a visibility of Secchi disc. (30 cm diameter). Chemical parameters included phosphate (PO_4), total inorganic nitrogen (TINIT) = nitrate (NO_3) + nitrite (NO_2) + ammonium (NH_4), and silicate (SiO_4), and were analysed in accordance with STRICKLAND & PARSONS (1972).

Chlorophyll *a* (Chl*a*) was determined from 500-mL sub-samples filtered through Whatman GF/F glass-fibre filters stored at $-20^\circ C$. These were homogenized and extracted in 90% acetone for 24 h at room temperature (HOLM-HANSEN *et al.*, 1965). Samples were analysed fluorometrically with a Turner TD-700 Labora-

tory Fluorimeter (Sunnyvale, CA) calibrated with pure Chl *a* (Sigma). The detection limit for chlorophyll *a*, considering the filtered volume of water was 0.02 µg/L.

Phytoplankton samples were preserved in neutralized formalin (2.5% final concentration) and observed with an Olympus IX-71 inverted microscope according to the Utermöhl method (UTERMÖHL, 1958). Sub-samples (50 mL) were settled for 24-48 hours in counting chambers (Hydro-Bios) before analysis. Results are expressed as number of cells per litre (abundance). Counts of microphytoplankton (cells >20 µm, MICRO) were done at a magnifications of 400x in one central transect, 200x in 2-3 central transects and at 100x in the whole of the counting chamber base plate to obtain the most accurate evaluation. Nanophytoplankton (cells 2-20 µm, NANO) were counted in 30 randomly selected fields-of-view at magnification 400x.

Zooplankton samples were preserved with a 2.5% final concentration of formaldehyde neutralized with CaCO₃. Samples were sedimented for 24 h in plastic containers in the laboratory, from which about 3/4 of the water was decanted. The remainder was poured into a glass cylinder (10 cm diameter), and was sedimented for a further 24 h after which the superfluous volume was decanted. This process reduced the original volume of 5 l to 30 ml in 72 hours. Decanting was accomplished using a vacuum pump and a slightly curved pipette (KRŠINIĆ, 1980). The organisms were counted using an Olympus IMT-2 inverted microscope at magnifications of 100 and 400x. Total samples were counted in a glass cell, dimensions 7 x 4.5 x 0.5 cm. Our study focused on taxopodids (TAX), non-loricate ciliates (NLO), tintinnids (TIN), rotatorians (ROT), nauplii (NAU), calanoids (CAL), cyclopoida-oithonids (OIT), cyclopoida-oncaeids (ONC), harpacticoids (HAR), bivalve larvae (BIV) and appendicularians (APP).

The Pearson's correlation coefficient (R_p) was used for correlations between all variables. Parameters were compared between groups using t-test.

RESULTS

Environmental characteristics and phytoplankton

Hydrography, nutrients and phytoplankton performed during the 2002 and 2003. were analysed in detail by ČALIĆ *et al.*, (2013). Therefore, in this paper we have analyzed only some general aspects: minimum, maximum and average for investigated parameters which were presented in Table 1. The highest annual temperature variability was in the layer from the surface to 6 m depth, with minimum values in January and maximum in July. The water column is isothermal during the periods February/March, and summer August/September. The greatest variability of salinity was noted in the surface layer. In January, April/May and October values were between 29 – 34, depending on the rainfall and the influence of the Neretva River. Below 10 m depth salinity values were higher than 38 with a maximum of 38.62 in February. Sea water is well saturated with oxygen, with the exception of layer below 6 m depth, with minimum values of 0.64 recorded in July. Nitrate (NO₃⁻) predominated in total inorganic nitrogen (TINIT) with higher values in the layer 0-6 m depth. The nitrite (NO₂⁻) concentration was low, but slightly higher in January and February in the deeper layers. Also the ammonia (NH₄⁺) concentration was higher below 6 m deep with a maximum in July.

Phosphate (PO₄³⁻) concentration was low, while silicates (SiO₄⁴⁻) were high in January and October, the period of minimum impact of the Neretva River. Higher values of Chl_a concentrations were noted in the period March/April and August. The annual variability of nano and microphytoplankton are shown in Fig. 9, as a percentage of the maximum average value. The nanophytoplankton maximum average value of 7.4×10^5 cellsL⁻¹ was in April 11th and microphytoplankton of 1.0×10^4 cellsL⁻¹ in April 17th, 2002. During the winter and spring abundance of nanophytoplankton was rarely less than 60% of the maximum value. There was a sharp decline in abundance in early July, and

Table 1. Annual range (MIN, MAX) and average (AVG) values of physical, chemical and phytoplankton parameters at Usko station in 2002. For phytoplankton abundance data from 2003 were also included.

Parameter	MIN	MAX	AVG
Temperature (T°)	6,9	24,8	15,98
Salinity	29,08	38,62	37,05
Sigma -t	20,91	29,51	27,14
Transparensy (Secchi, m)	4,5	12	8,16
O ₂ saturation (%)	0,64	1,45	1,02
NO ₃ (µmol/L)	0,01	9,88	0,81
NO ₂ (µmol/L)	0,01	0,85	0,17
NH ₄ (µmol/L)	0,11	1,03	0,44
PO ₄ (µmol/L)	0,01	0,49	0,04
SiO ₄ (µmol/L)	0,67	15,81	3,97
Chl <i>a</i> (µg/L)	< DL*	1,83	0,55
Total microphytoplankton (cells/L)	1770	324300	10577
Total nanophytoplankton (cells/L)	56632	2818048	742992

*Below the detection limit (see Material and Methods)

only 5% abundance was found in early January 2003. Microphytoplankton predominated in April, May and January 2003, while the rest of the year abundance varied between only 17 and 30% of the maximum value. Dinoflagellates greatly predominated in the total abundance of microphytoplankton.

Zooplankton composition and abundance

Taxopods

Sticholonche zanclea was the only species of this group of protists found in the Bay. Usually it is present in late fall (Table 2). It is rare in surface layers, and occasionally very numerous from 5 m to the bottom. In late January 2003 it was present with a maximum abundance of 38 ind.L⁻¹ at 10 m depth.

Non-loricates

In the Bay of Mali Ston, 8 species of planktonic non-loricate ciliates were noted. Only one species, *Zoothamnium pelagicum* is of colonial form (Table 2). All species were present in the winter period, when they predominated (Fig. 2). Several smaller forms could not be determined because of deformities caused by the pre-

servatives used. Some organisms can be identified only as living specimens. Non-loricates are with a maximum of 1584 ind.L⁻¹, March 27th at 9 m depth. The average annual value was 127±178 ind.L⁻¹. Positive significant correlation was with ONC, BIVA, TINT, CHL*a*, MICRO, NANO; R_p = 0.134 - 0.295, and negative correlation with CAL, OIT, APP, PO₄ and TEM, -0.340; p < 0.05, n = 395 (Table 3).

Tintinnids

Tintinnids are characteristic ciliates in the plankton of Mali Ston Bay. In the period from January 2002 to January 2003, 27 species of tintinnids was recorded. A maximum of 14 species occurred on July 2002, after which the number of species dropped (Table 2, Fig. 3). Estuarine-neritic species are characteristic, while open-sea species immigrate into Mali Ston Bay, carried by movements of the bottom waters. These include *Dadayiella ganymedes*, *Codonella aspera*, *Petalotricha ampulla*, and *Rhabdonella spiralis*.

The frequent investigations carried out in the year 2002 clearly showed two distinct populations, a winter and summer one (Fig. 4). The first peaks in abundance occurred in the middle of February. High densities were recorded for all layers from the surface to the bottom. The domi-

Table 2. List of species during investigation at station Usko in the Bay Mali Ston from January 2002 to January 2003.

	Jan.	Feb.	Mar.	Apr.	May	Jun..	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.
Taxopoda													
<i>Sticholonche zanclea</i>	+	+	+	+	+						+	+	+
Nonloricate ciliates													
<i>Laboea strobila</i>	+	+	+	+		+			+	+	+	+	
<i>Lohmanniella appendiculariformis</i>		+	+	+									
<i>Myrionecta rubra</i>						+	+		+				
<i>Strombidium capilatum</i>		+	+	+									
<i>Strombidium conicum</i>	+	+	+						+	+			
<i>Strombidium</i> sp.	+	+	+	+	+						+		
<i>Tontonia ovalis</i>	+		+	+						+			
<i>Zoothamnium pelagicum</i>											+	+	
Undetermined species	+	+	+	+	+	+				+	+	+	+
Tintinnida													
<i>Codonella aspera</i>	+												
<i>Tintinnopsis campanula</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Tintinnopsis compressa</i>				+		+	+	+	+	+			
<i>Tintinnopsis cylindrica</i>									+				
<i>Tintinnopsis fennica</i>							+	+	+				
<i>Tintinnopsis fracta</i>								+	+	+	+	+	+
<i>Tintinnopsis karajacensis</i>	+		+	+	+	+			+	+			
<i>Tintinnopsis levigata</i>		+		+		+		+		+			+
<i>Tintinnopsis nana</i>	+	+	+	+		+	+	+					
<i>Tintinnopsis radix</i>	+	+	+	+	+	+	+			+	+	+	+
<i>Codonellopsis schabi</i>	+	+	+					+	+	+		+	+
<i>Stenosemella nivalis</i>	+	+	+							+	+	+	+
<i>Stenosemella ventricosa</i>	+	+	+	+	+		+	+	+	+	+	+	+
<i>Helicostomella subulata</i>	+	+	+	+	+	+	+	+	+	+			
<i>Metacylis joergenseni</i>						+		+		+			
<i>Petalotricha ampulla</i>	+			+									
<i>Favella ehrenbergii</i>					+								
<i>Favella serrata</i>	+	+		+									
<i>Rhabdonella spiralis</i>					+	+							
<i>Dictyocysta elegans</i>	+		+	+	+	+	+		+	+			
<i>Amphorides quadrilineata</i> var. <i>minor</i>										+	+	+	
<i>Dadayiella ganymedes</i>										+			
<i>Steenstrupiella steenstrupii</i>			+	+									
<i>Eutintinnus apertus</i>							+	+	+				
<i>Eutintinnus fraknoi</i>					+	+	+	+	+				
<i>Eutintinnus lusus-undae</i>							+	+	+	+			
<i>Eutintinnus tubulosus</i>					+	+							
Rotatoria													
<i>Synchaeta neapolitana</i>	+		+	+	+								
Copepoda													
Calanoida													
<i>Paracalanus parvus</i>				+	+	+	+	+	+	+			
<i>Temora stylifera</i>						+	+	+	+	+	+		
<i>Temora longicornis</i>						+		+					
<i>Diaxis pygmoea</i>									+				

<i>Centropages kroyeri</i>						+	+	+	+	+			
<i>Centropages typicus</i>			+	+			+			+			
<i>Ctenocalanus vanus</i>			+	+		+							
<i>Isias clavipes</i>	+										+		+
<i>Acartia clausi</i>	+	+	+	+						+	+	+	+
Cyclopoida													
<i>Oithona nana</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Oithona similis</i>	+		+	+		+						+	+
<i>Paroithona parvula</i>	+	+		+									
<i>Monothula subtilis</i>	+	+	+	+	+	+				+	+	+	+
<i>Oncaea waldemari</i>	+	+	+	+	+					+	+	+	+
<i>Oncaea zernovi</i>	+	+	+	+	+							+	+
<i>Oncaea vodjanitskii</i>													+
<i>Spinoncaea ivlevi</i>	+	+	+										+
Harpacticoida													
<i>Euterpina acutifrons</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Microsetella norvegica</i>		+	+						+	+	+	+	+
Other metazoans													
Bivalve larvae	+	+	+	+	+	+	+			+	+		
Gastropoda larvae					+	+							
Appendicularians						+	+	+	+	+			
Total number of taxa	30	26	31	32	22	27	20	20	24	31	20	19	20

Table 3. Pearson's correlation coefficients of the zooplankton assemblages, phytoplankton and physico-chemical parameters ($N=395$, significant at $p<0.05$.): non-loricate ciliates (NLO), tintinnids (TIN), nauplii (NAU), calanoids (CAL), oithonids (OIT), oncaeids (ONC), harpacticoids (HAR), bivalve larvae (BIV), appendicularians (APP), temperature (TEM), salinity (SAL), NO_3 , NO_2 , NH_4 , PO_4 , SiO_4 , CHLa , microphytoplankton (MICRO) and nanophytoplankton (NANO).

	NLO	TIN	NAU	CAL	OIT	ONC	HAR	BIV	APP	TEM	SAL	NO_3	NO_2	NH_4	PO_4	SiO_4	CHLa	MICRO
NLO																		
TIN	0.295																	
NAU	-0.037	0.464																
CAL	-0.108	0.209	0.534															
OIT	-0.159	0.327	0.726	0.639														
ONC	0.134	0.245	0.177	0.054	-0.065													
HAR	0.082	0.203	0.266	0.265	0.295	0.281												
BIV	0.221	0.178	0.057	0.026	0.006	0.393	0.225											
APP	-0.171	0.130	0.397	0.341	0.551	-0.337	0.172	-0.027										
TEM	-0.340	-0.075	0.345	0.407	0.504	-0.483	0.146	-0.163	0.488									
SAL	0.082	0.455	0.386	0.219	0.326	0.363	0.265	0.316	0.021	-0.036								
NO_3	0.008	-0.181	-0.231	-0.262	-0.306	-0.035	-0.031	-0.203	-0.100	-0.204	-0.489							
NO_2	0.042	0.134	0.081	-0.047	-0.041	0.614	0.115	0.041	-0.270	-0.439	0.196	0.133						
NH_4	-0.049	0.161	-0.113	-0.134	-0.207	0.058	-0.115	-0.145	-0.180	0.268	-0.096	0.138	0.047					
PO_4	-0.164	-0.004	0.150	0.123	0.151	-0.036	0.071	-0.167	0.180	0.156	-0.066	-0.026	0.071	-0.028				
SiO_4	-0.033	0.229	0.194	0.218	0.201	0.204	0.261	0.044	0.092	-0.045	-0.038	0.430	0.188	0.135	0.094			
CHLa	0.219	-0.034	-0.111	-0.087	-0.172	-0.013	-0.231	0.068	-0.094	-0.151	-0.039	-0.142	-0.230	-0.088	-0.032	-0.144		
MICRO	0.161	-0.184	-0.056	-0.091	-0.139	-0.062	-0.113	-0.048	-0.062	-0.001	-0.106	-0.163	-0.125	-0.147	0.028	-0.252	0.381	
NANO	0.185	0.171	-0.130	-0.181	-0.223	0.370	-0.091	0.302	-0.275	-0.326	0.191	-0.209	0.052	0.122	-0.138	-0.078	0.309	0.188

Bold; Significant correlations, $p<0.05$

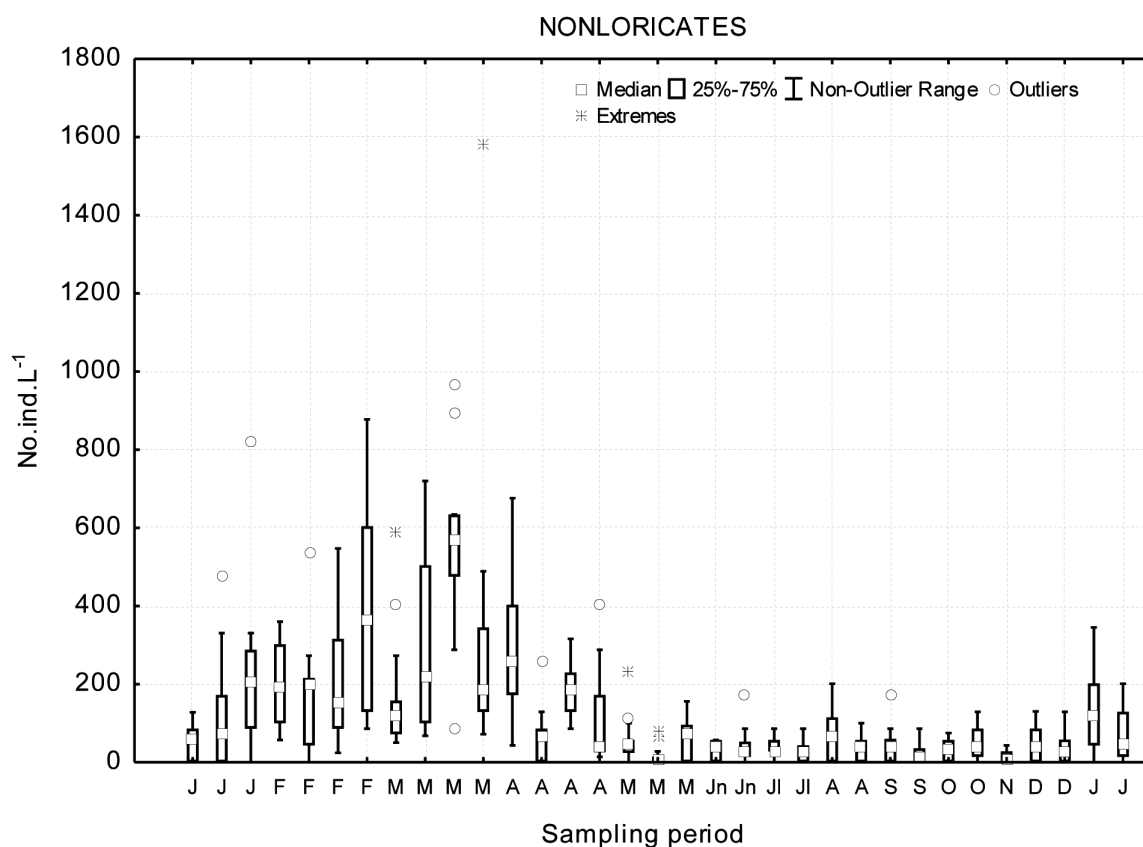


Fig. 2. Nonloricates annual distribution of abundances (No.ind.L⁻¹).

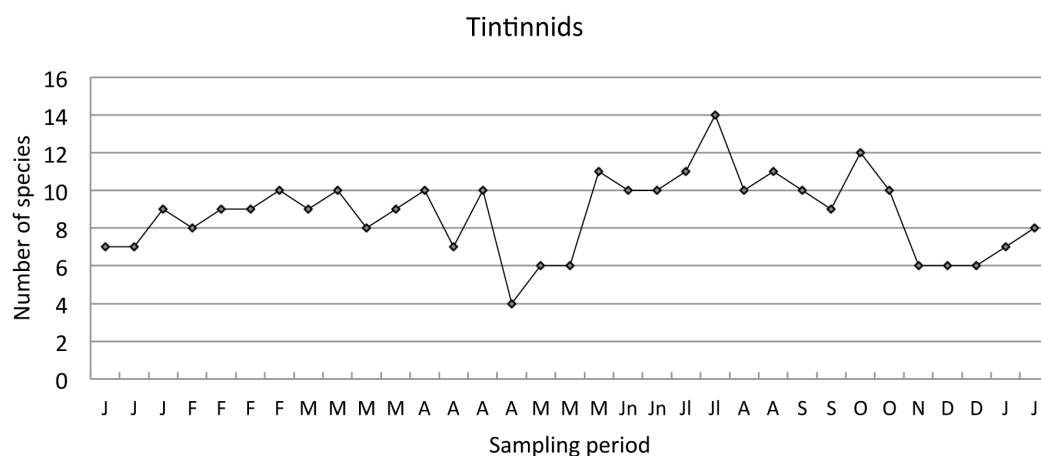


Fig. 3. Annual distribution of tintinnid species.

nant species *Tintinnopsis nana* reached a density of 1096 ind.L⁻¹ at 3 m and similar at 11 m depth. The next sample, taken in February, showed a significant drop in density values of both upper layers. High average values were retained only at the bottom, also due to the dominance of the above-mentioned species. For samples taken in

March, the predominant species was *Helicos-tomella subulata*, which had a maximum value of 520 ind.L⁻¹ at 4 m depth and 528 ind.L⁻¹ at 13 m depth. The summer tintinnid population began to increase in July, with an annual average maximum in abundances occurring around August (Fig. 4). The increased density value

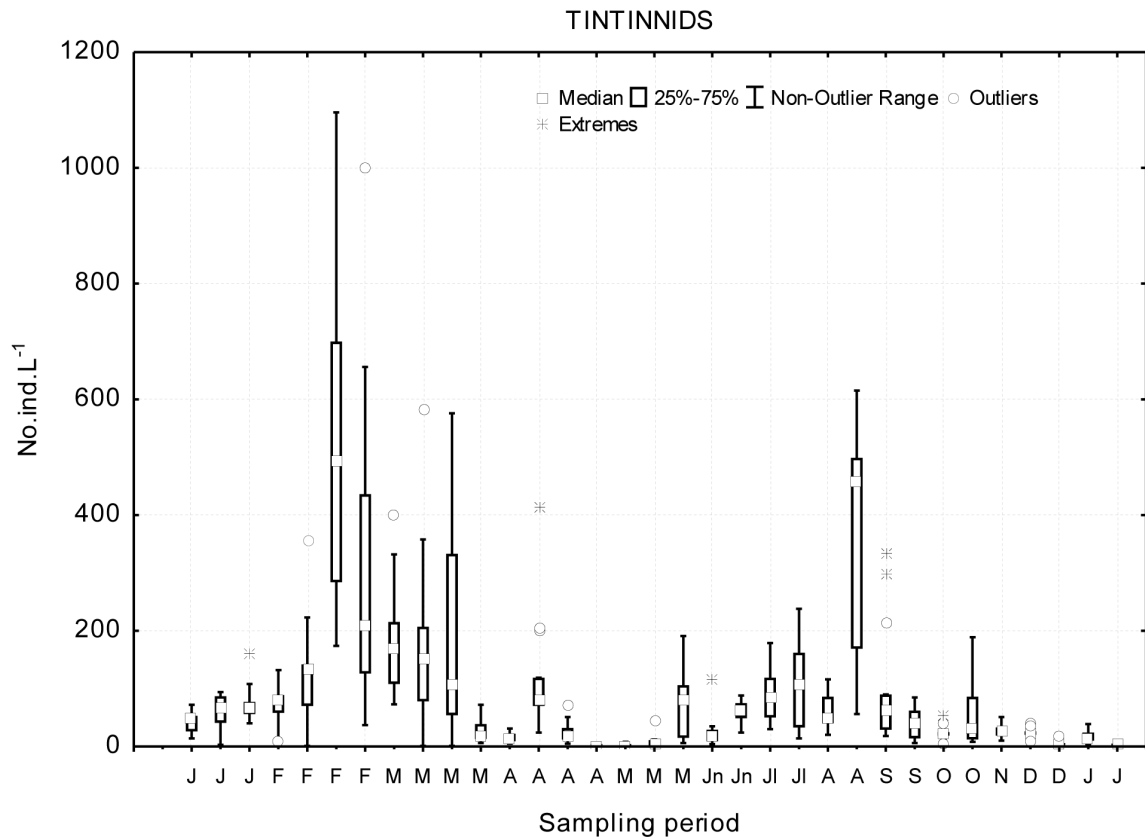


Fig. 4. Tintinnids annual distribution of abundances (No.ind.L⁻¹).

was followed by species diversity observed on August 30th (Fig. 3). Generally tintinnids were most abundant in bottom layers from 9 to 13 m depth, while they were only occasionally the most abundant species at depths of 0.5 to 3 m. Significant differences between upper layer (0-6 m) and lower layer (7-13 m) were found for the whole period (t-test; $p < 0.01$, $n = 238$), and with positive significant correlation ($R_p = 0.637$; $p < 0.05$). Positive significant correlation was with all zooplankton assemblages, ($R_p = 0.130 - 0.464$, $p < 0.05$, $n = 395$), with chemical parameters and nanophytoplankton, especially salinity ($R_p = 0.455$) (Table 3).

Rotatorians

Rotatorians appeared sporadically during the winter and spring, while they disappeared from plankton populations during early summer. Only the species *Synchaeta neapolitana* was recorded. Greatest abundance was recorded in

early January 2002, with the maximum of 35 ind.L⁻¹ at 9 m depth. Significant correlation with all other assemblages was not found.

Copepod assemblages

This paper separately treats nauplii, the earliest developmental stages of copepods and copepod assemblages: calanoids, oithonids, oncaecids and harpacticoids.

Copepod nauplii

Copepod nauplii were the most abundant planktonic metazoans. They are constantly present during the winter and spring with a relatively low abundance, while very numerous during the summer and early fall (Fig. 5). A maximum of abundance was 466 ind.L⁻¹ at the end of August at 9 m depth (annual average 49.8 ± 67.6 ind.L⁻¹ $n = 476$). Typically during the warm part of the year stratification of abun-

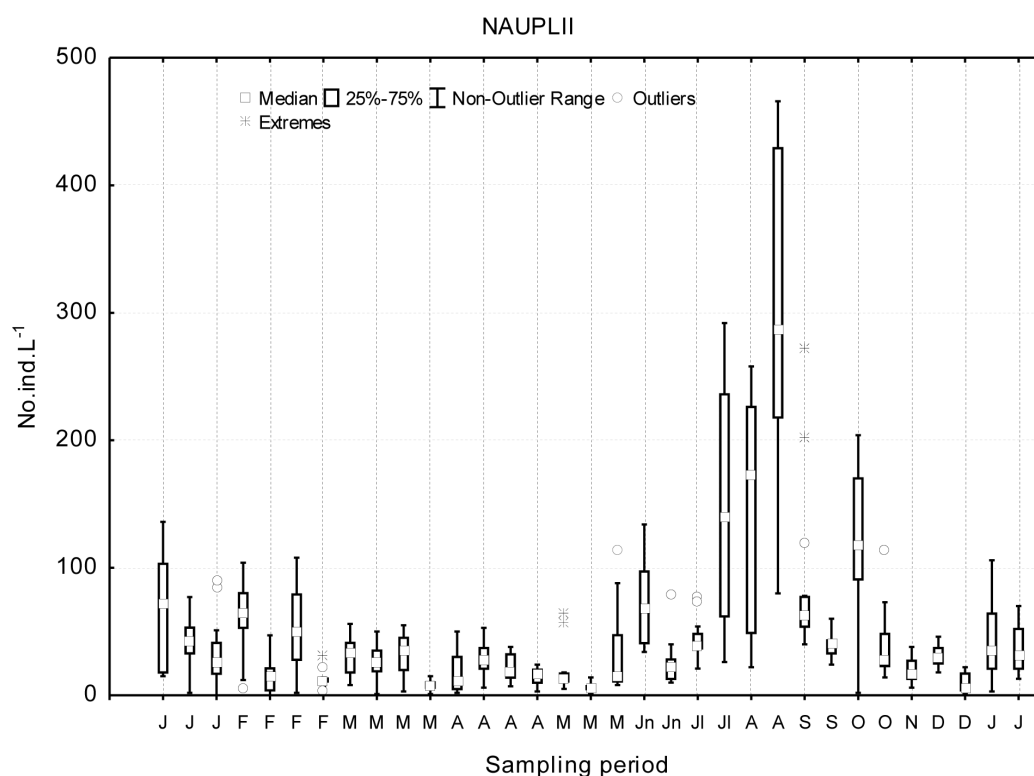


Fig. 5. Nauplii annual distribution of abundances (No.ind.L⁻¹).

dances is to be found, with the lowest values at the surface layer, up to 4 m depth. Significant differences between the upper layer (0-6 m) and the lower layer (7-13 m) were found during whole period (t-test; $p < 0.001$, $n = 238$). The highest positive significant correlation was with CAL and OIT, $R_p = 0.534-0.726$, and higher with TEM and SAL, $R_p = 0.345-0.386$; $p < 0.05$, $n = 395$ (Table 3).

Calanoids

Copepodites and calanoid adults have an annual distribution very similar to nauplii, however with more than 10 times lower values. They make up only 12.5% of the total number of post-naupliar copepods. After very low abundance during winter, the number slightly increases in April, while the maximum is during the end of August (Fig. 6). A maximum of abundance was 31 ind.L^{-1} , (annual average $3.17 \pm 4.39 \text{ ind.L}^{-1}$, $n = 476$). In the total abundance of calanoids more than 80 % were copepodites. Stratification of calanoids was very pronounced in July and

August, when the majority of the population is below 3 m depth. Significant difference (t-test, $p < 0.05$, $n = 238$) between the upper layer (0-6 m) and the lower layer (7-13 m) was found throughout the whole period. Moreover, weak correlation was found between the layers ($R_s = 0.376$; $p < 0.05$). Of adult calanoids, estuarine-neritic species were present, while *Paracalanus parvus* and *Acartia clausi* predominated during warmer part of the year. A highest positive significant correlation was found with OIT, $R_p = 0.639$, $p < 0.05$, $n = 395$. Positive significant correlation was with HAR and APP, as well as with SAL, PO_4 , SiO_4 , while negative correlation with NO_3 , NH_4 and NANO (Table 3).

Oithonids

Oithonids also have a similar distribution as nauplii and calanoids, and were especially numerous during the warm season (Fig. 7). Their abundance was 70% lower than the average values for nauplii. Oithonids were the most numerous and account for approximately 60%

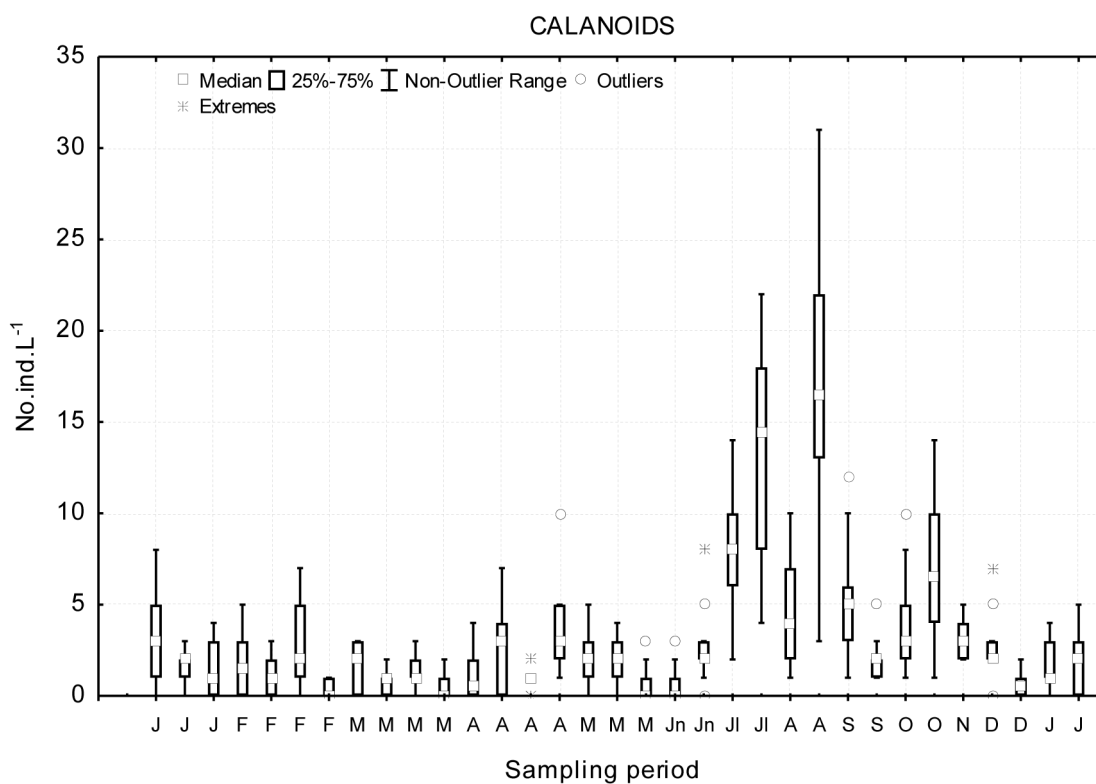


Fig. 6. Calanoids annual distribution of abundances (No.ind.L^{-1}).

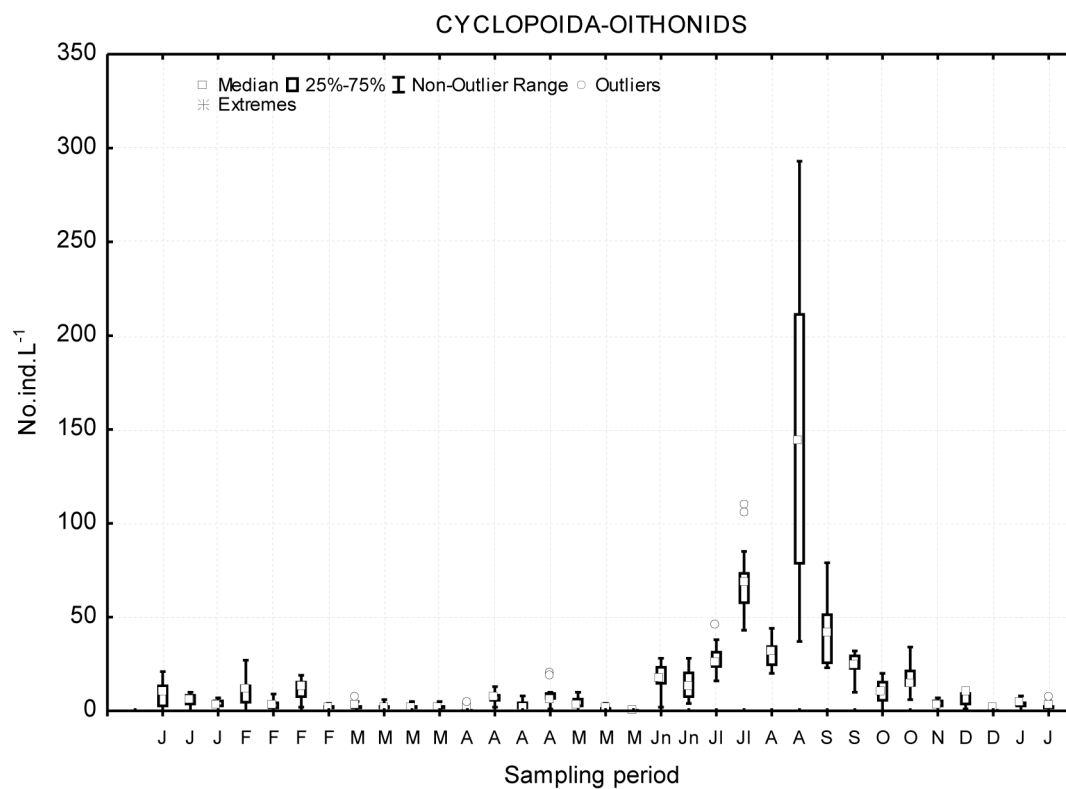


Fig. 7. Oithonids annual distribution of abundances (No.ind.L^{-1}).

of the total number of post-naupliar copepods. A maximum abundance was 293 ind.L⁻¹, (annual average 15.08±31.6 ind.L⁻¹, n=395). In total abundance of oithonids, more than 90 % were copepodites. Stratification of oithonids was pronounced, and a significant difference between the upper layer (0-6 m) and the lower layer (7-13 m) was found (t-test; $p < 0.01$, n=238), also with significant correlation ($R_p = 0.682$; $p < 0.05$). Three species of oithonids were noted, *Oithona nana* that particularly predominated through the period investigated, *Oithona similis* that was present in low abundance during the winter and *Paroithona parvula* that was noted during January and February, but only above the sea bottom at 12 or 13 m depth. The highest positive significant correlation was with APP, $R_p = 0.551$, $p < 0.05$, n=395. Also high positive correlations was with TEM, $R_p = 0.504$, and negative with NO₃, NH₄, CHL_a, MICRO, NANO (Table 3).

Oncaeiids

Carnivorous oncaeids were present in the cold seasons (Fig. 8). Oncaeids were numerous and accounted for approximately 24.7 % of the total number of postnaupliar copepods. A maximum abundance was 84 ind.L⁻¹, on January 31st, 2003 at 10 m depth (annual average 6.3 ± 12.2 ind.L⁻¹, n = 476). In total abundance of oncaeids, about 94 % were copepodites. Stratification of oncaeids was pronounced and a significant difference between the upper (0-6 m) and lower layer (7-13 m) was found (t-test; $p < 0.001$, n=238), with significant correlation ($R_p = 0.737$; $p < 0.05$). A positive significant correlation was with BIV, $R_p = 0.393$, $p < 0.05$, n=395, negative with APP, $R_p = -0.337$, $p < 0.05$, n=395. Also positive correlation with HAR, SAL, SIO₄ and NANO, the highest with NO₂ $R_p = 0.614$, while negative significant correlation with APP and

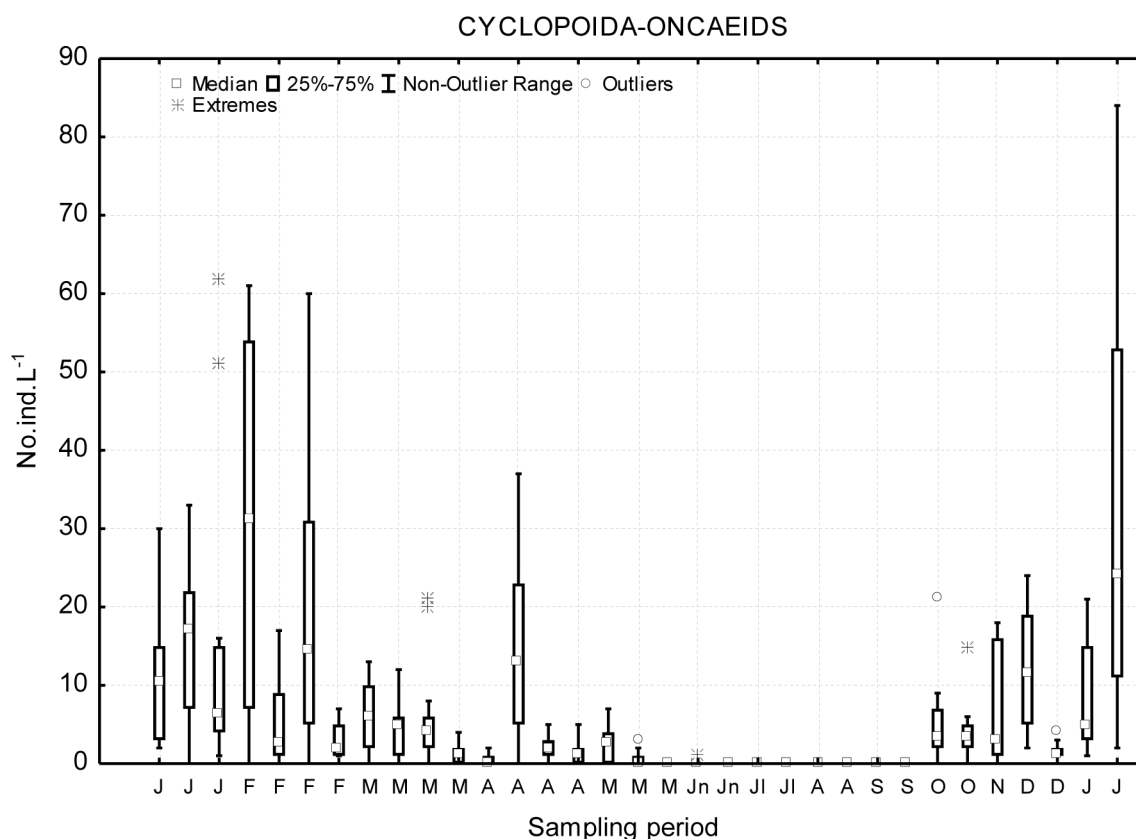


Fig. 8. Oncaeids annual distribution of abundances (No.ind.L⁻¹).

TEM, (Table 3). Five species of oncaeids were noted. *Monothula subtilis* and *Oncaea walde-mari* as dominant coastal water species, while *Oncaea zernovi*, *O. vodjanitskii* and *Spinoncea ivlevi* were typical representatives in the subsurface layer of the open sea.

Harpacticoids

Harpacticoids showed a similar distribution to oncaeids during the research period, but with a much lower abundance (Fig. 9). Their average proportion was only 3.8 % of the total number of postnaupliar copepods. A maximum abundance was 16 ind.L⁻¹, on January 31st 2003 at 13 m depth (annual average 0.97 ± 1.8 ind.L⁻¹, n=395). A low positive significant correlation was with ONC, $R_p = 0.281$, $p < 0.05$, n=395 (Table 3). Common species was *Euterpina acutifrons* during spring and autumn, while low abundance species *Microsetella norvegica* were present in winter mainly close to the sea bottom.

Bivalve larvae

Bivalve larvae were occasionally observed to be numerous, while they disappeared from plankton during the warm season (Fig. 10). The most pronounced peaks were in February and then in late March and April and again in the fall. A maximum abundance was 50 ind.L⁻¹, on March 27th at 10 m depth. Stratification of larvae was pronounced, with the bulk of the population below 6 m depth. A low positive significant correlation was noted with ONC, $R_p = 0.393$, as well as SAL and NANO, $p < 0.05$, n=395 (Table 3). Bivalve larvae, belonging to the species *Ostrea edulis*, are cultivated in the Bay.

Appendicularians

Appendicularians were present during the summer and early fall (Fig. 11). A maximum abundance was 10 ind.L⁻¹, on August 30th, at 9 m depth. More than 90% of individuals were

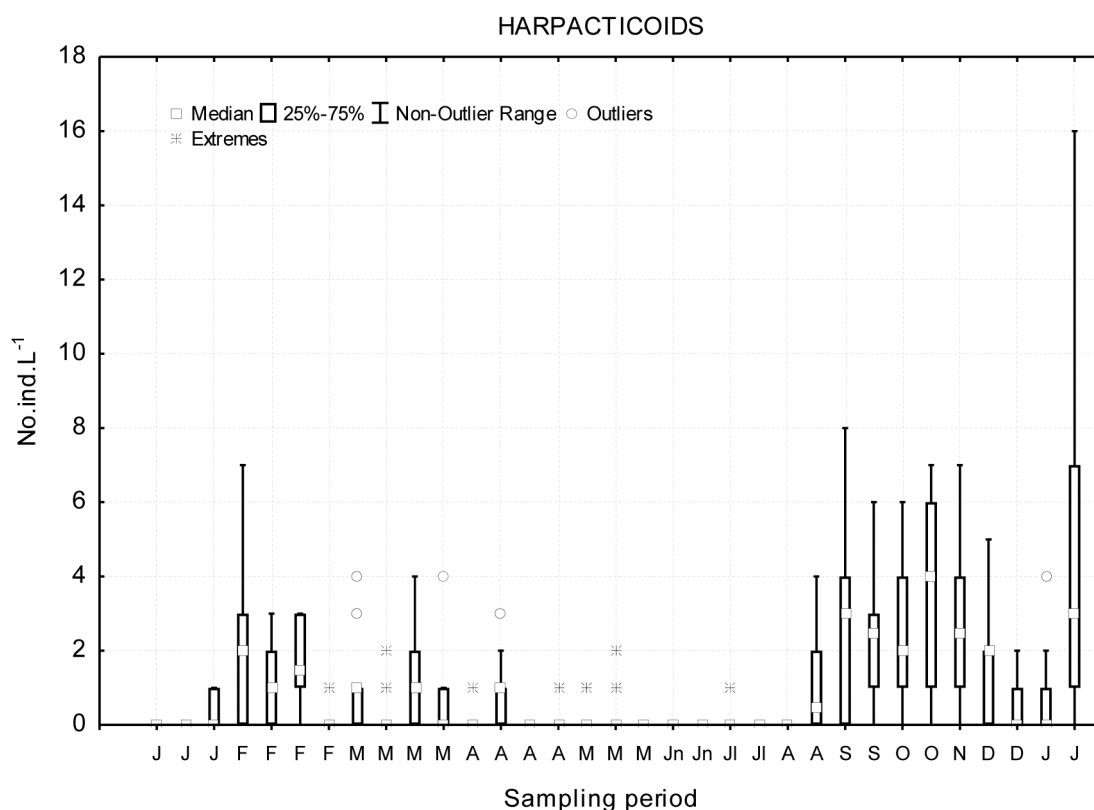


Fig. 9. Harpacticoids annual distribution of abundances (No.ind.L⁻¹).

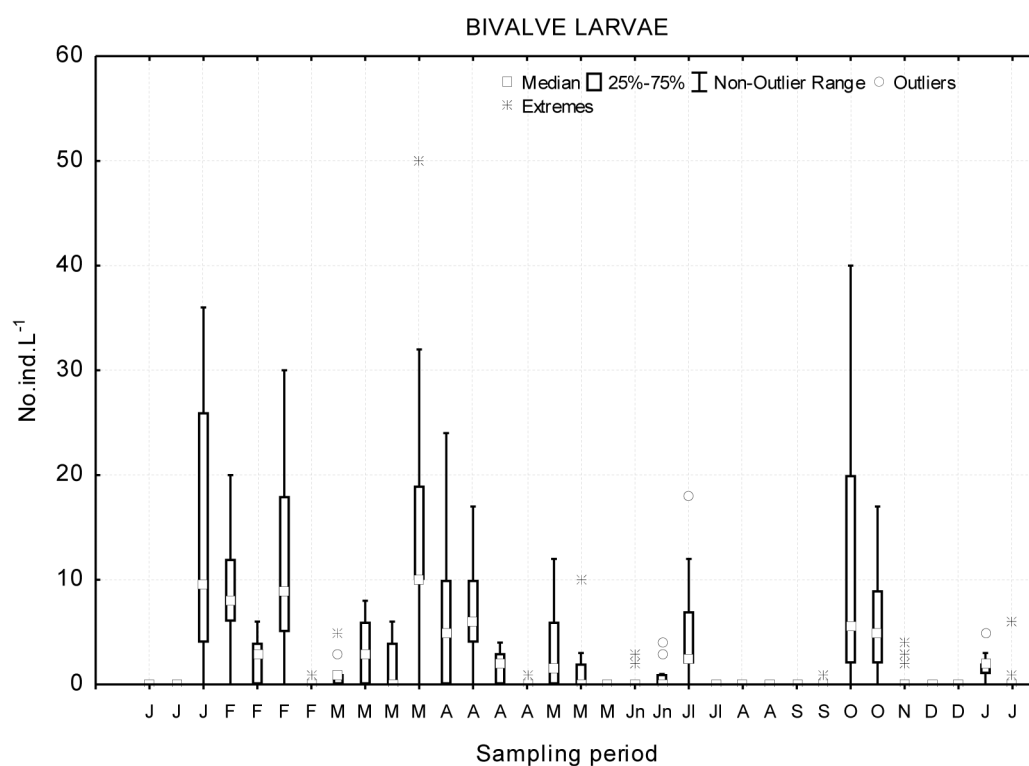


Fig. 10. Bivalve larvae annual distribution of abundances (No.ind.L⁻¹).

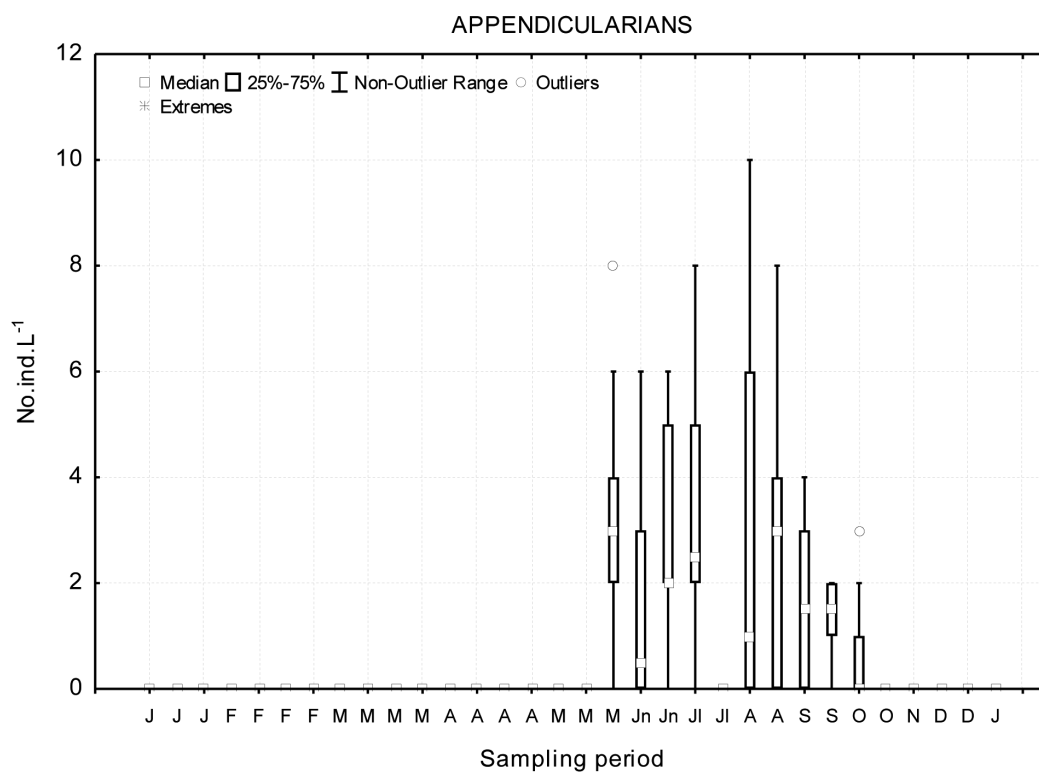


Fig. 11. Appendicularians annual distribution of abundances (No.ind.L⁻¹).

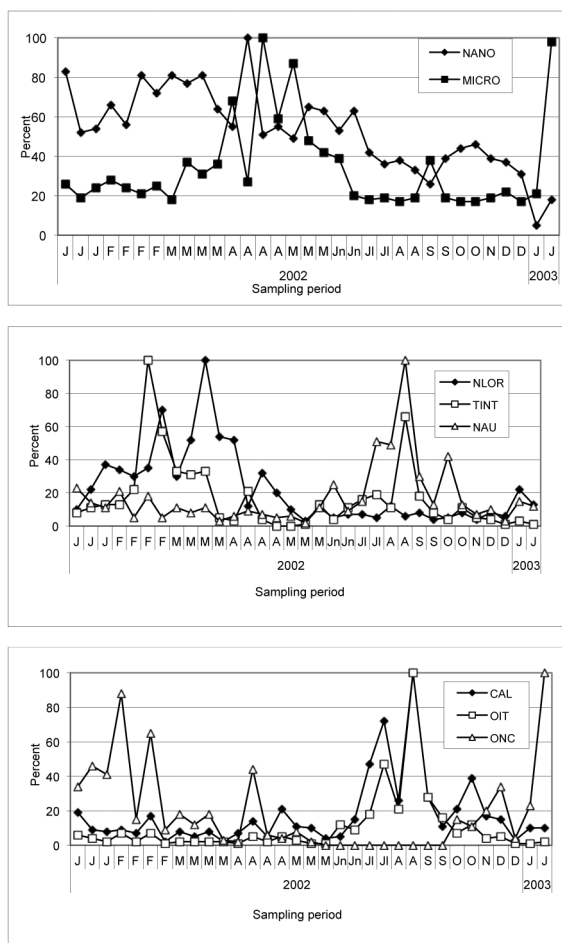


Fig. 12. Annual succession of nano and microphytoplankton, neloricates, tintinnids, nauplii, calanoids, oithonids and oncaeids. Values present percentages of an average maximum population density (No. ind.L⁻¹) for annual period. Average maximum values are: NANO 7.4×10^5 , MICRO 1×10^4 , NLOR 575, TINT 552, NAU 292, CAL 17.9, OIT 151, ONC 34.

juvenile forms. In the population, *Oikopleura dioica* was the predominating species. The bulk of population was below 6 m depth. A positive significant correlation was found with NAU, $R_p=0.397$ and OIT, $R_p=0.551$, and the highest correlation with TEM, $R_p=0.488$, and low negative correlation with ONC, $R_p=-0.337$, $p < 0.05$, $n=395$ (Table 3).

DISCUSSION

Sampling consideration

The methodology of zooplankton sampling is still a major problem, especially in shallow coastal ecosystem. By using a plankton net, it is not possible to get a real quantitative data. HARRIS *et al.*, (2000), have very well documented these problems associated with net samplings. Many authors have investigated a small fraction of zooplankton using a water bottle of various volumes (STICKNEY & KNAWLES, 1975; SOROKIN, 1977; KRŠINIĆ, 1980; REVELANTE & GILMARTIN, 1992; VIDJAK *et al.*, 2006). While the mesozooplankton in the neritic area were collected using plankton net WP-2 with mesh netting of 125 to 200 μm (TRANTER & SMITH, 1968). In previous studies, the qualitative-quantitative composition of mesozooplankton, its vertical distribution and abundance in the Bay of Mali Ston use was made of an “Adriatic Trap” (KRŠINIĆ, 1990; LUČIĆ & KRŠINIĆ, 1998). In the Bay of Mali Ston a volume of 5 litres enables an estimate of the abundance of neritic microzooplankton, including a majority of the taxonomic groups (KRŠINIĆ, 1980), as confirmed by this study for protozoans, oithonid, oncaeid, harpacticoid copepods, calanoid copepodites, rotatorians, larvae and juvenile forms of other metazoans. In this paper we have recorded only 9 adult species of calanoids, unlike 22 (ONOFRI, 1986) and 25 species (LUČIĆ & KRŠINIĆ, 1998). Accordingly, the volume used is not suitable for qualitative-quantitative research of larger and rarer zooplankton organisms.

Diversity, abundance and seasonal succession of zooplankton assemblage in the Mali Ston Bay

The diversity of all zooplankton assemblages in the Mali Ston Bay on an annual and multiannual basis is primarily dependent on hydrographic conditions. Mostly estuarine-neritic species are present in the bay. However, during the autumn/winter period with high salinity in the bottom layer open-sea species migrate from the southern Adriatic. During unfavourable conditions, estuarine tintinnids form cysts, as

do many species of ciliates (REID & JOHN, 1978; KRŠINIĆ, 1987b). These cysts drop to the bottom and remain there until conditions are satisfactory for a new plankton phase. Some species develop only after a few years, besides immigration was more pronounced in some particular years, such as in 1983 (KRŠINIĆ, 2010a).

Open-sea species reproduce for a while in the Bay and then disappear from the plankton. Females of typical open-sea oncaeid species *Oncaea zernovi* in January and February have egg sacks, and among plankton there are a higher percentage of their copepodites. During previously investigations in summer the number of tintinnid species was lowest towards the inner region of the Bay (KRŠINIĆ, 1987a). However, during the summer of 2002 open-sea tintinnids were present, which is in accordance with the increase in salinity throughout the water column (ČALIĆ *et al.*, 2013). *Sticholonche zanclea* were present in the bay at the time of their dominance in coastal water during autumn and winter, and only the rotatorian species *Synchaeta neapolitanum* were observed in the winter of 2000 with lower salinity in surface layer. Annual distribution of bivalve larvae and juvenile appendicularians is in accordance with previous studies (VILIČIĆ *et al.*, 1994; LUČIĆ & KRŠINIĆ, 1998).

Annual succession of zooplankton abundance for characteristic assemblages in the Bay of Mali Ston is shown in Figure 12. Typically, non-loricate ciliates, tintinnids and oncaeid copepods predominate in the winter and spring, while nauplii, oithonids and calanoid copepods predominate during the summer. During the summer-autumn period with calanoid and oithonid domination there is negative correlation between microphytoplankton and the majority of zooplankton were found. The increase of nanophytoplankton abundance was positively correlated with the increase of non-loricates, onceaeids and tintinnids, and negatively with the increase of calanoids and oithonids. The nauplii, calanoids and oithonids showed positive significant correlations with temperature and salinity, but negative with phytoplankton. Non-loricates showed negative correlation with temperature, which indicates their abundant winter-spring

distribution together with phytoplankton. Tintinnids had positive correlation with salinity while Oncaeids had weak correlation with temperature and some investigated environmental factors.

The findings are in accord with previous research in this area (KRŠINIĆ, 1979, 1987a; KRŠINIĆ & MUŠIN, 1981; RUDENJAK-LUKENDA, 1990; LUČIĆ & KRŠINIĆ, 1998). Inter-annual variability in abundance of zooplankton assemblages is dependent on hydrography and productivity conditions. Basis research on tintinnids showed that their maximum abundance occurred in the period from February to April with values of 250-1850 ind.L⁻¹ (KRŠINIĆ, 2010b). Although the abundance of non-loricates and tintinnids in the Bay is very high during their usual dominance in the plankton, these values are up to 7 times lower for non-loricates and 5 times lower for tintinnids than in areas with high eutrophication of anthropogenic origin in the western part of the northern Adriatic and bays of the eastern Adriatic coast, such as the Šibenik Channel and Vranjic Basin in the Kaštela Bay. In addition, unlike the Mali Ston Bay, in eutrophicated areas the maximum abundance of ciliates occurred in the summer or early fall (REVELANTE *et al.*, 1985; KRŠINIĆ, 1995, 2007; BOJANIĆ *et al.*, 2005).

A maximum population value for the species *Tintinnopsis levigata* and *Helicostomella subulata* of 1830 ind.L⁻¹ was recorded on March 1983. *T. levigata* was scarce according to the most recent investigations carried out from 2002 to 2003, while the dominant species were *Tintinnopsis nana* in the middle of February and *Helicostomella subulata* in March. The summer tintinnid population is typically absent at the furthest end of Mali Ston Bay. However, tintinnids were numerous in this region during the summer of 2002. Specifically, the summer tintinnid population showed a lower abundance but a significantly greater biovolume than in winter (winter maximum 12.1 x 10⁶ µm³ x L⁻¹, summer maximum 30.97 x 10⁶ µm³ x L⁻¹), which was the result presence of larger, dominant species, such as *Codonellopsis schabi*, *Tintinnopsis cylindrica*, *Stenosemella ventricosa*, and *Eutintinnus fraknoi* (KRŠINIĆ, 2010b).

Adult copepods and their developmental stages are the most numerous metazoans in the

Bay of Mali Ston plankton. In the Bay were present representatives of major orders of planktonic copepods such as calanoids, cyclopoids and harpacticoids. According to the annual or multi-annual study of abundances variability, nauplii predominate very much with higher values compared to the copepodites and adult individuals. Their abundance reaches a very high value of 466 ind.L⁻¹, which is comparable to the most productive bays of the eastern Adriatic coast (REVELANTE *et al.*, 1985; KRŠINIĆ, 1995; KRŠINIĆ *et al.*, 2007; BOJANIĆ *et al.*, 2005; VIDJAK *et al.*, 2006). Two annual peaks of post-naupliar copepods are evident: the first from February to April at the period of oncaeid dominance, and the second in July or August due to the dominance oithonid and calanoids copepods. *Oithona nana* is the most numerous copepods during the period of warmer sea with an abundance of 15 ind.L⁻¹ or even 290 ind.L⁻¹ for copepodites in August 2002. During the period of lower sea temperatures, species of the genus *Oncaea* are predominant. In January 2003, the abundance of copepodites genus *Oncaea* reached the value of 84 ind.L⁻¹, while in March of the same year was 100 ind.L⁻¹. Adult species of *Monothula subtilis* reached 20 ind.L⁻¹ (unpublished data). From typical estuarine-neritic calanoids, only three species are extremely dominant with more than 65% of the total number of adult calanoids, with a maximum abundance in summer (30 ind.L⁻¹). Although planktonic harpacticoids are important in the coastal sea, in the Bay they show a low abundance of up to 16 ind.L⁻¹.

The structure of copepods in the Mali Ston Bay is specific and differs significantly from all other populations in the bays and channels along the eastern Adriatic coast. Compared with other bays in the eastern Adriatic coast regardless of their trophic levels only in the Mali Ston Bay were found as typical sub-surface species oncaeids from the deep southern Adriatic (KRŠINIĆ, 1998). During periods of low temperature <15 °C, and high salinity > 37, oncaea copepodites adults achieve very high abundance, and together with non-loricate ciliates, tintinnids and planktonic harpacticoids the winter-spring zooplanktonic community

comprises: *Tintinnopsis nana*, *Helicostomella subulata*, *Monothula subtilis* and *Synchaeta neapolitana*. On the other hand, during the summer post-naupliar oithonids are highly dominant, with abundance significantly higher than observed in other bays. (KRŠINIĆ & VILIČIĆ, 1989; BOJANIĆ *et al.*, 2005; KRŠINIĆ, 1995, 2007; KRŠINIĆ *et al.*, 2007; VIDJAK *et al.*, 2009). At the same time in the Bay were recorded high abundances of post-naupliar calanoids and oithonids, juvenile appendicularians and summer tintinnids. We can thus conclude that during the warmer period with temperatures > 20 °C and high salinity > 37 summer-autumn zooplanktonic communities comprised: *Codonellopsis schabi*, *Stenosemella ventricosa*, *Oithona nana* *Paracalanus parvus*, *Acartia clausi*, *Oikopleura* sp. During the transition period between the two zooplankton communities the greatest abundances were of nano and microphytoplankton (Fig. 12), because at that time the lowest level of “top-down” control of phytoplankton is from zooplankton grazing pressure.

In the Mali Ston Bay were also present non-crustacean assemblages such as rotatorians, appendicularians and bivalve larvae. Rotatorians were present in the colder part of the year, but were of quantitative importance only from the beginning of January with very low salinity values < 34 in the surface layer. It is probable that with a relatively high salinity in the Bay rotatorians were considerably less quantitatively important, as, for example, in the Ombla Estuary (CARIĆ *et al.*, 2012), the lower Neretva river estuary (JASPRICA *et al.*, 2012), or the Etang de Tau, France (LAM-HOAI *et al.*, 1997). Appendicularians were present only during the summer, when the largest percentage consisted of juvenile exemplars with abundance <10 ind.L⁻¹. Other bays along the eastern coast of the Adriatic show a similarly high abundance of appendicularians. However, in a few cases in the eutrophicated areas of the Šibenik Channel abundances were extremely high, in August 2001, at 5 m depth, was recorded a maximum of juvenile appendicularians of 830 ind.L⁻¹. (KRŠINIĆ, 2007).

Trophic state of the Mali Ston Bay and implications for the shellfish cultivation potential

The inner part of the Mali Ston Bay is traditionally used for the production of *Ostrea edulis*, amounting to about 2 million units/year and *Mytilus galloprovincialis* about 3,000 t/year (BENOVIĆ, 1997). In addition, in the Bay live ten species of shellfish in different population densities which are exploited for food (ŠIMUNOVIĆ, 1981). The abundance and distribution of bivalve larvae has been recorded by several authors, who mention numbers in the order of 50 ind. L⁻¹ (MOROVIĆ & ŠIMUNOVIĆ, 1980; VILIČIĆ *et al.*, 1994), which is also confirmed by this current research. However, previous studies have shown that there are oyster larvae in the plankton between April and December, with a peak in the warmer periods of the year from May to September. During the present investigations bivalve larvae were not recorded between July and October. As can be seen from Figure 10 the increase in plankton abundance in March, April and October can be connected with two intense periods of oyster spawning. From January to March, the connection is presumably with *Mytilus* larvae.

The trophic status of the Mali Ston Bay is not easy to define. Based on the abundance of phytoplankton (VILIČIĆ *et al.*, 1998) the area was classified as a moderately naturally eutrophicated ecosystem. While the latest research (ČALIĆ *et al.*, 2013), using the TRIX index for classification of the coastal areas, which takes into account concentration of chlorophyll *a*, the dissolved oxygen, dissolved inorganic nitrogen and total phosphorus (KARYDIS, 2009), the Bay is of only low trophic level, or oligotrophic.

To offer a definition of trophic status on the basis of zooplankton populations in coastal waters requires no elaborate classification index. However, comparison of the number of species and abundance of dominant zooplankton assemblages between the oligotrophic southern Adriatic and the Bay of Mali Ston does not confirm that they have the same trophic status. In the southern Adriatic were recorded three times

the number of tintinid species and four times of copepod species compared to the Bay (KRŠINIĆ, 2010b; HURE & KRŠINIĆ, 1998). On the other hand, abundances of tintinnids were up to 30 times greater and for copepods 3-15 times greater in all developmental stages than in the surface layer (50 – 0 m) of the southern Adriatic.

The Bay of Mali Ston is distinct from the typical estuary, where the constant river flow continuously enriches the area with nutrients. Relatively constant high salinity, especially in the layer below 6 m depth, is confirmation of a significant inflow of fresh water during the rainy season both from submarine springs “vrulje” and from the Neretva River. As a result, the concentration of nutrients is not significantly different from the values found in the open sea (ČALIĆ *et al.*, 2013). We can assume that in the Bay the processes of bio-deposition and excretion are of particular importance, specifically as a result of the cultivation of oysters and mussels in the Bay, since a richness of benthic filter feeders produces very large quantities of faeces and pseudofaeces. According to ITO & IMAI (1955) in a study of Japanese waters calculated that a raft of oysters 60 m² would annually produce 0.6 to 1 metric tonnes (dry weight) of faecal material. Individual oysters deposited 1.62 g/week with a September maximum of 3.92 g/week. It has also been estimated that the organic content of bio-deposits will vary between 9 to 22.1% dry weight (HAVEN & MORALES-ALAMO, 1966).

It would be necessary in the Mali Ston Bay to determine the remineralization of organic detritus and excretion by filter feeders organisms, including all zooplankton. The ammonia generated by oysters is taken up by rapidly regenerating phytoplankton in the water column (PIETROS & RICE, 2003). In the Mali Ston Bay we find the classical food chain but also, and perhaps even more important, there is also the microbial food chain. During the whole year, calanoid copepods are dominant filter feeders, as are appendicularians and partially oithonid copepods in summer. The food chain initiates the degradation of organic detritus through the action of heterotrophic bacteria, non-loricated ciliates, tintinnids, harpacticoids and probably

oncaeid copepods during winter/spring and oithonids in the summer/autumn period. The variation in abundance of non-associated bacteria in the Bay in 1988 were from 3.3×10^5 to 8.6×10^6 cells ml^{-1} , with a maximum in March (RADONIČIĆ, 1992). The predominant species *Oithona nana* has a considerable power of adaptation to food variety because they are able to consume a much wider range of foods than the other copepods and have a low metabolic rate. Their consumption may include suspended detrital organic material (LAMPITT & GAMBLE, 1982). Non-calanoid copepods, predominantly omnivores (oithonids, oncaeids, harpacticoids), have the role of trophic link between microbial and traditional food web (ABERLE *et al.*, 2010).

In the Bay of Mali Ston, tintinnids are a very important food resource for oysters in the autumn-winter period when oysters are preparing to spawn (KRŠINIĆ, 1987b). According to a recent study (PEHARDA *et al.*, 2012) other smaller zooplankton assemblages were also important, however, it was found that oncaeid copepods formed a significant element in the diet of juvenile sardines when present in this part of the bay (Kršinić, unpublitate data). Based on the above population of planktonic protists and small metazoans, we can conclude that the Mali Ston Bay shows meso-oligotrophic characteristics. Since zooplankton assemblages vary considerably according to their mode of feeding and annual succession, their role in the food web is crucial for maintaining stability in the pelagic community of the Mali Ston Bay.

According to long-term research in the Bay, it was found that the abundance of almost all assemblages were below 5 m depth. This dis-

tribution is determined by hydrographic conditions, and by the intense filtering of shellfish in aquaculture farms that have been growing in the surface layer to a depth of about 4 m. While the shellfish culture reduced phytoplankton and smaller zooplanktonic factions, on the other hand organic detritus significantly enriches the water column. It is necessary to consider the question of the capacity of the bay for the cultivation of shellfish and fish aquaculture, and to be aware of its potential to disturb the stability of the system. For these issues additional research is required and must take into account all the specific peculiarities of the bay with including quantification of substance flows at all trophic levels. Due to the fact that previous studies have demonstrated a decreased abundance of most zooplankton assemblages from the Usko station towards the inner part of the bay, being the area of most intense aquaculture (KRŠINIĆ, 1987b), it is important to carefully consider any possible increase in the capacity for shellfish farming.

For this very specific area along the eastern coast of the Adriatic, larger areas should be reserved for cultivation of the European oyster *Ostrea edulis*, whereas a decrease of other organisms farming should follow.

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Struktura populacija planktonskih protista i malih metazoa u Malostonskom zaljevu (Jadransko more) - pokazatelji za utvrđivanje trofičkog stuplja i potencijala za uzgoj školjkaša

Frano KRŠINIĆ^{1*}, Marijeta ČALIĆ² i Marina CARIĆ²

¹*Institut za oceanografiju i ribarstvo, Šetalište Ivana Meštrovića 63, 21000 Split, Hrvatska*

²*Sveučilište u Dubrovniku, Institut za more i priobalje, Kneza Damjana Jude 12, 20000 Dubrovnik, Hrvatska*

**Kontakt adresa, e-mail: fkrsinic@izor.hr*

SAŽETAK

Uzorkovanja u Malostonskom zaljevu provedena su u razdoblju od siječnja 2002. do siječnja 2003. na postaji Usko, jednom tjedno ili dva puta mjesečno, s 5L Niskin crpcem u slojevima od 1 m od površine do 13 m dubine. Istraživale su se populacije planktonskih protista i malih metazoa, kao što su: taxopodidi, nelorikatnih cilijati, tintinidi, rotatorii, od kopepoda, nauplii, kalanoidi, oitonidi, onceidi, harpacticoidi, ličinke školjkaša i juvenilne apendikularije. Raznolikost i bogatstvo svih skupina na godišnjoj razini u prvom redu ovisi o varijabilnostima hidrografskih parametara. Tijekom razdoblja niskih temperatura i visokog saliniteta, onceidni kopepoditi i odrasli su vrlo brojni, a zajedno s nelorikatnim cilijatima, tintinidima i planktonskim harpaktikoidima čine zimsko-proljetnu zooplanktonsku zajednicu. Tijekom toplijeg razdoblja u uvjetima visokih temperatura i slanosti je ljetno-jesenska zooplanktonska zajednica, koju čine postnaupliarni kalanoidi i oitonidi, nedorasle apendikularije i ljetni tintinidi. Iz brojnosti planktonskih protista i malih metazoa može se zaključiti da je Malo Stonski zaljev mezo-oligotrofnih karakteristika. U radu se raspravlja o važnosti biodepozicije fekalnog materijala školjki koje se uzgajaju u zaljevu i o mogućem negativnom utjecaju na ekološku ravnotežu zbog mogućeg značajnog povećanje kapaciteta školjkaša u uzgoju.

Ključne riječi: Jadransko more, zaljev Malog Stona, raspodjela, zooplankton